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SUMMARY

The study of the dry rot disease of corn caused by *Diplodia zeae* shows it to be a prevalent disease in Iowa, resulting in losses, the past two seasons, ranging from 3 to 15 percent of the ears at harvest and a 11 percent damage to the seed corn. The loss in stand from diseased seed in many fields amounted to 15 percent. A still further loss results from nodal infection and weak plants grown from slightly infected seed.

Infected seed either does not germinate at all or produces weak plants. However, the fungus is not systemic and does not grow into the plant from infected seed.

Infection is local, not systemic. The fungus may attack the plant thru the silk and tips of ears but the nodes and the ear shanks are the chief points of attack. At the time of flowering the leaf sheaths become loosened from the stalk forming a pocket for the collection of pollen, moisture and spores of *Diplodia zeae*. Within this cavity the fungus attacks the sheaths and nodes. Under conditions of excessive moisture within the sheath, sugar is excreted. The presence of this sugar may also favor dry rot infection. The fungus may spread readily in seed corn while it is curing or in corn cribs in damp, rainy weather.

Heavy rainfall at the end of the growing period very materially favors the development of *Diplodia*.

The temperature for the growth of the mycelium of *Diplodia zeae* on media is as follows: Minimum about 15°; optimum 30°; and maximum 35°C. It will not grow in the absence of oxygen.

The use of cellulose by the fungus enables it to readily penetrate a modified rag doll germinator, or to weaken the nodes of the stalk. The use of cellulose moreover explains the capacity of the fungus to live in the soil on old stubble.

In experiments on roots of seedlings and older plants it has been possible to produce only slight infection.

Seedling blight caused by *Diplodia zeae* is the result of infection of the ear the previous year. Planting of badly infected seed results in decreased stand and obviously a lower yield. The viable seed from diseased ears produces weak plants. *Diplodia* infected seed is difficult or impossible to detect except by germination tests.

A long rotation, the early field selection of seed, and seed germination tests in the spring are probably the most practical means of control.



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FIG. 1. SYMPTOMS OF DIPLODIA INFECTION ON LEAF SHEATHS.

DRY ROT OF CORN

BY L. W. DURRELL

Dry rot of corn has been known since 1834 and study has been given in the past to losses caused by its action on the ears and to its supposed agency in stock poisoning. Its pathological effect on the corn crop, however, has been seriously neglected. As a result some of the symptoms of the disease are either imperfectly understood or attributed to other causes, such as the so-called "root rot." Little is known of the development of dry rot or of the mode of infection of the causal organism, *Diplodia zeae*, and there is a prevailing idea that moldy corn or discolored stalks are the result of the organism working up thru the roots from the soil or from diseased seed. The association of dry rot with the discoloration of the sheaths after flowering is important in the consideration of the disease, throwing light on the method of infection by the organism.

The unusual prevalence of dry rot in Iowa the past two seasons (1921 and 1922), coupled with conditions governing its parasitism and its significant effect on seed corn and field stand induced the author to make a detailed study of the disease in relation to corn growing under Iowa conditions.

DISTRIBUTION AND LOSS

Dry rot caused by *Diplodia zeae* occurs annually in Iowa, but becomes of serious importance only when favorable weather conditions prevail. An epidemic of the disease occurred in 1921 thruout the central and northeastern parts of the state where there was heavy rain during the late summer and early fall. Little dry rot was found in the drier western part of the state along the Missouri River. This epidemic was the first recorded for the state since 1909.

In 1922 dry rot was prevalent in parts of the state, but the distribution differed from that in 1921. The greatest amount occurred southwest of the central portion of the state, the north and eastern sections being quite free of dry rot. Where local storms injured the crop during the summer, dry rot was prevalent, and particularly was this the case where hail fell.

The writer is greatly indebted to Dr. I. E. Melhus for many helpful suggestions during the progress of this work and in the preparation of this manuscript. He also wishes to acknowledge the helpful criticism of Dr. J. C. Gilman.

Altho certain areas in the state in 1921 were quite free from dry rot, other sections showed 20 percent of the ears partially or wholly destroyed. The badly infected ears were largely left in the field. It was estimated for the whole state that the loss was four percent or 17,767,000 bushels. This loss does not take into consideration the decreased yield due to dry rot injury on the stalks and seed corn. The former is very difficult to determine. Its importance may be judged somewhat by its prevalence on the stocks in the field. Counts were made in many different fields in five counties as follows:

Story county, 47 percent of stalks infected.
 Hamilton county, 25 percent of stalks infected.
 Webster county, 31 percent of stalks infected.
 Marshall county, 11 percent of stalks infected.
 Warren county, 4 percent of stalks infected.

That this amount of *Diplodia* had a tendency to reduce the yield can hardly be questioned. In fact, subsequent data indicate that under certain conditions *Diplodia* on the stalks may reduce the yield 10 percent.

In addition, the damage to seed corn is readily overlooked by the corn grower. Some idea of the amount of seed corn killed by *Diplodia* may be had from the following germination data: of 650 ears collected in the north and east central part of the state 11 percent were unfit for seed purposes. In another collection consisting of 5,000 ears gathered from representative sections of the state, the same average, 11 percent, were found unfit for planting. Out of 130 ears collected in the south central part, 7 percent were infected with *Diplodia*. As this corn was quite representative of the state, it gives a fair index of the distribution and prevalence of *Diplodia zeae* as affecting seed ears.

The losses in the state from dry rot infection, based on 1921 statistics, may be summed up as follows: An average loss of 4 percent of the crop at harvest, or 17,767,000 bu. of moldy corn left in the field. In addition, 11 percent of the seed ears unfit to plant, approximately 141,499 bu. This latter is a double loss, both as feed and seed. This diseased corn does not sprout and the resulting stand is thereby decreased. In many fields, only a 75 percent stand was obtained, which resulted in an abnormal reduction in yield.

Further, the dry rot infection on the stalks injures the plant sufficiently to reduce the yield. In bad cases this amounted to about 10 percent.

DESCRIPTION AND SYMPTOMS

The previous descriptions of dry rot have been made by Burrill and Barrett (1) and by Heald and Wilcox (2), attention was given only to mycelial growth and fruiting on the ears and stalks. The earlier symptoms of the disease on the sheaths and nodes were not mentioned. In other papers by Burrill (3, 4) some of these symptoms were associated with a bacterial disease of corn. He writes of these symptoms as follows:

"The first indication of the disease in a field.....is the dwarfed condition of the young plants.....In many cases it is upon the lowest ground.....Upon closer observation it is found that young diseased plants besides being smaller.....are uniformly yellowish in color.....The bottom portion of the stalk is likewise affected.....If split longitudinally the inner tissue of this lower part is seen to have a uniform dark color..... After midsummer, especially, the disease becomes apparent thru discolorations of the sheaths. These leaf sheaths become variously spotted as observed from the exterior. Occasionally there is a reddish color."

SYMPTOMS OF DRY ROT CAUSED BY *DIPLODIA ZEAE*

On leaf sheaths: On the leaf sheaths the fungus produces reddish or purplish spots of varying size and shape, appearing after flowering of the corn plant. These lesions may extend down into the node of the stalk or up the leaf killing and discoloring the midrib (fig. 1).

On the ears: *Diplodia zeae* readily attacks the ears. The badly diseased ears may be completely covered with the mycelium, giving a white moldy appearance when the husk is removed. This may be accompanied by discoloration, the ear being grey or dirty looking, even brown or blackish. In some cases the fungus may penetrate the husk so as to be readily observed from the outside, often fruiting profusely on the husk. In other instances the fungus may attack only the tip or the butt end of the ear. The latter condition is most prevalent. Often the infection is scarcely visible as a fine web of mycelium between the kernels at the base of the ear; even this may be lacking and only in shelling the kernels can a fine white film be seen at the base. When the kernels show such symptoms the cob and chaff also have a dusty appearance and the kernels are usually loose.

On breaking an ear badly affected with *Diplodia zeae*, the fungus is often found fruiting profusely on the cob. The fruiting bodies appear as small black specks.

On the kernels the fungus may show dusty or dirty grey (fig. 2), in some instances fruiting on the crowns of the kernels. Sometimes the fungus is not visible and is only detected when the corn germinates, altho shrunken kernel tips appear to be quite constantly associated with dry rot infection.

On germination: The fungus developes in a few days from the infected kernel as a slightly cream colored cottony mass (fig. 2).

On stalks: *Diplodia zeae* attacks other parts of the corn plant besides the ear and sheaths. The shank of the ear is frequently affected (fig. 3) and white wefts of mycelium may be seen on it. More common, however, is the appearance of large numbers of fruiting bodies at the shank and nodes. In some cases the breaking of the shank is due to the presence of *Diplodia*, tho not always. On the stalk the symptoms are much like those on the shank. Within the sheath, at its base, and around the node may often be found a white growth of the mycelium. This may extend over the internode as well. Infection at the nodes, particularly the lower ones, is often manifested as a water soaked discoloration (fig. 4).

In general, however, *Diplodia* is most evident on the stalk as fruiting bodies which first emerge as minute whitish, evenly scattered dots, later becoming black (fig. 5). Infection on the stalk may occur at any node but especially on the lower ones and is sometimes accompanied by breaking at the weakened nodes.

GROWTH REACTION OF *DIPLODIA ZEA*

MOISTURE RELATIONS

Only a brief survey of the action of *Diplodia zeae* is necessary to emphasize the importance of its relation to moisture. In fact, moisture is the determining factor in its growth. The moisture content of the substratum and not the atmospheric humidity limits the development of *Diplodia*. The fungus grows down from the nodes more rapidly than up, possibly due to the greater moisture supply. Corn, high in moisture, stored in warm weather is quickly overrun with *Diplodia zeae*, tho only slightly infected when harvested. It has been repeatedly observed that seed corn may be infected without showing evidence of *Diplodia*. When such slight infection is accompanied by high moisture the results are serious.

Tho *Diplodia* is scarcely noticeable or is even invisible, on infected corn when picked, such ears when hung on the rack, if not quickly dried, may become badly molded by the fungus. Care should be given to the rapid drying of seed



FIG. 2. CORN KERNELS SHOWING DIPLODIA INFECTION.

The figure to the left shows badly diseased kernel as taken from the ear. The figures in the center and to the right illustrate the appearance of diseased kernels after several days in germinator. The fungus usually develops as a fluffy white mass of mold on diseased kernels.

corn, particularly if picked early, so as to prevent any growth of *Diplodia* that may be on the ears. To supplement observations on the spread of dry rot on seed ears, 25 ears were picked before frost and hung in wire racks. On the base of



Fig. 3—Corn shank infected with *Diplodia*. The fungus is fruiting profusely at base of shank.



Fig. 5—Infected corn node showing pycnidia of *Diplodia*.

each cob was placed a fragment of *Diplodia mycelium*. In a week these ears showed a growth of *Diplodia* an inch or more up from the butt. Twelve other ears treated the same way, but wrapped up in oil cloth became entirely overgrown with *Diplodia mycelium*.

In the field the relation of moisture to *Diplodia zeae* is very noticeable. A correlation exists between the amount of dry rot and increased precipitation. In 1921 this relation showed plainly. The map (fig. 6) gives the areas of greatest precipitation during the month of August, 1921. Heavy rains fell that month, between August 15 and 31. July and the first part of August were dry. Surveys showed more *Diplodia* infection on the ears in the areas of heavy rainfall than in the drier sections. In the western part of the state, where the precipitation was light, little dry rot occurred.

In 1922 a similar condition was noted, tho not so pronounced. Fig. 7 shows the precipitation for August. The prevalence of dry rot on the ears indicated that here again the *Diplodia* development coincides with the areas of greatest precipitation. In Hardin county there occurred 2.5 percent of dry rot, in Henry county 4.5 percent and in Marion and Dallas counties 7.5 percent. Southward thru Polk and Warren counties the percentage of dry rot decreased from 10 to 4 percent. In Sioux county 3.5 percent of dry rot was found, this low figure in a relatively wet area being explained by the fact that this section of the state had its lowest precipitation during early September. Corn was less advanced in this northern locality and the heavy rains in August occurred too early to combine with other factors favoring dry rot.

Altho conditions may vary from year to year, the observations of the past two seasons rather indicate that it is the amount of rainfall during August that determines the amount of dry rot. Altho the bulk of the precipitation occurred at the end of the growing period in 1921 and 1922, there were times earlier in the season when moisture was apparently sufficient to initiate infection. The inference is therefore, that certain other conditions must also accompany high precipitation in order to produce an epidemic of dry rot. These other conditions exist annually toward the end of the growing season and are as follows: First there is a maximum of stored food in the corn plant; second the rapid growth of tissues has ceased and the leaf sheaths have become loosened; third, the loose leaf sheaths afford lodgment for *Diplodia zeae*. The presence of water between the leaf sheaths and the stalk at this time initiates infection. In seasons when a plentiful water supply is available during this period (August), dry rot is prevalent.

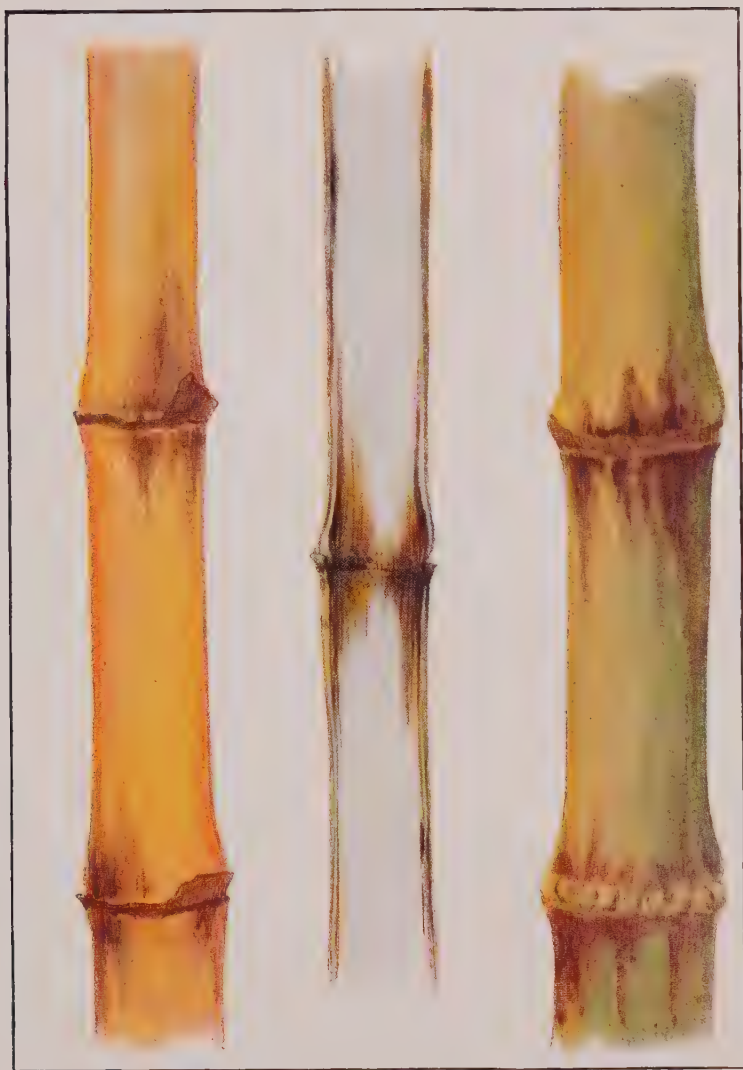


FIG. 4. SYMPTOMS OF DIPLODIA ZEA AT BASE OF CORN STALK.
The sheaths are torn away to show darkened, water soaked areas at nodes. The
central figure is a section of the stalk showing diseased
area following outer rind.

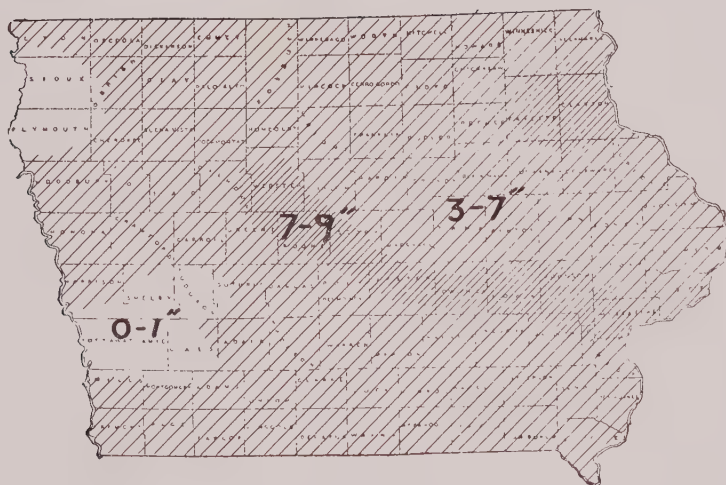


Fig. 6—Rainfall in Iowa 1921.

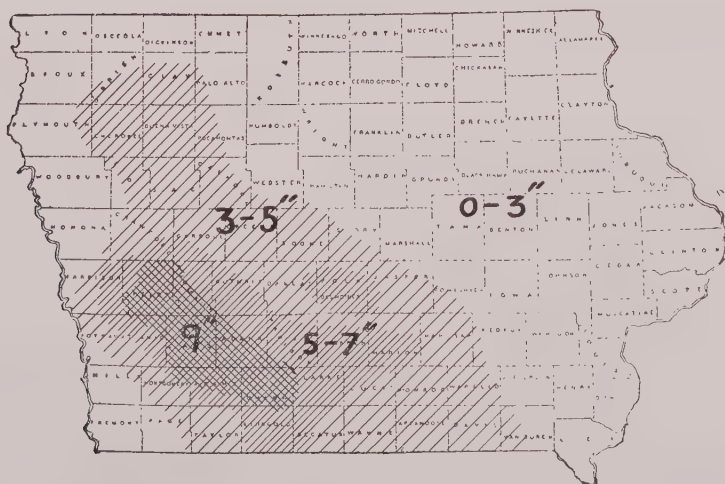


Fig. 7—Rainfall in Iowa 1922.

TEMPERATURE RELATION

Important as moisture may be in the development of dry rot, temperature also has a significant relation. The effect of temperature on the growth of *Diplodia zeae* was studied on Pfeffer's solution to which had been added five percent dextrose and one percent peptone. Flask cultures were used and the inoculum consisted of uniform bits of fungus averaging .016 gms.

After inoculation the cultures were held in electrically controlled ovens for a definite time, when the growth was removed, washed, and dried to constant weight.

The growth of *Diplodia zeae* at different temperatures in grams dry weight was as follows (Also see fig. 8):

No. cultures	...	10°C	15°C	20°C	25°C	28°C	30°C	33°C	35°C	40°
104	0 gms.	0.745	1.306	1.681	1.949	1.961	1.589	1.298	0

The above figures indicate that the minimum temperature for growth lies between 10°C and 15°C, the maximum between 35°C and 40°C and the optimum between 28° and 30°C. The growth of *Diplodia zeae* in 153 petri dish cultures under the same conditions gave the same indications as to temperature. This temperature, it may be noted, is near the optimum for the growth of corn, 34°C.

The relation of the air temperature at the time of dry rot infection does not appear to be as significant a factor in the development of the disease as does the amount of rainfall. Yearly weather records show the temperature for the period of *Diplodia* infection to be very constant and offer ample opportunity for favorable growth of the fungus. The records for rainfall however, show wide fluctuation. Years of high precipitation as 1921 showed prevalence of dry rot.

The relation of soil temperature to *Diplodia* infection is not very direct. Root infection in growing plants as shown in field tests is practically nil. On the germinating seed and seedling however, some injury may occur. Slightly infected seed can readily be injured before the plant establishes roots.

Soil temperatures as determined in different parts of the state are as follows:

MEAN SOIL TEMPERATURE 1921

	Mitchell county	Story county	Pottawattamie county
May	15.0°C	15°C	17.2°C
June	22.8	24.5	24.5
July	26.1	27.8	25.5
August	21.6	21.6	25.5

The average soil temperature during corn planting in the north, central, and even the south part of Iowa, is seen to be near the minimum for the growth of *Diplodia*.

At Ames over several years the temperatures for May are as follows:

	1917	1918	1919	1920	1921
Maximum	14.4	25.5	15.5	16.1	16.7
Minimum	13.4	11.6	13.9	11.6	13.4

Figures were obtained from records secured by the Iowa Truck Crop Section of the Experiment Station.

These figures indicate that the soil temperature in Iowa at the time of corn planting and during its early growth is not favorable for *Diplodia zeae*. The damage to seedlings

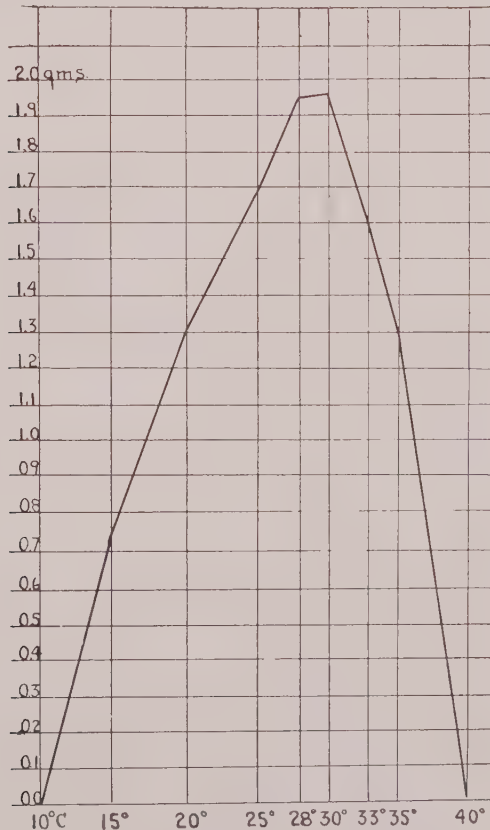


Fig. 8—Curve showing cardinal temperatures for growth of the fungus *Diplodia zeae*.

by this fungus occurs before the seed is planted when the grain is still on the cob. There, during the high temperatures of late summer, if moisture is sufficient, the dry rot fungus destroys the endosperm or injures the embryo so that when planted the seed is weak or dead. Some slight growth of the fungus can occur on the planted seed and set up a competition between *Diplodia zeae* and the seedling for the consumption of the stored food of the endosperm. The low soil temperature, however, makes this growth comparatively slow.

OXYGEN RELATIONS

Not only is the influence of moisture and temperature on the development of *Diplodia zeae* important, but also the relation of diminished oxygen supply. In order to determine this relation ten cultures of *Diplodia zeae* were held in an oxygen free chamber at room temperature for 13 days. The oxygen was removed by action of pyrogallie acid and sodium hydroxide. An equal number of cultures were held at the same temperature for the same length of time in a normal atmosphere. No growth was made on the cultures in oxygen free air, tho the checks grew profusely. At the end of the 13 days the cultures from one of the oxygen free chambers were removed and exposed to the normal atmosphere. In three days the fungus had completely covered these plates with a heavy growth, while the cultures left in the oxygen free chamber still showed no growth at the end of 18 days. *Diplodia* is apparently an aerobe.

These results are of particular interest in the light of the relation of *Diplodia zeae* to ensilage, which is cured under anerobic conditions. Since *Diplodia* requires oxygen for its growth and development, it is not a factor of importance on ensilage.

NUTRITIONAL REACTIONS

The relation of *Diplodia zeae* to its source of food supply is of interest, not only because of the destruction of the stored food in the corn kernel, but also because of the relation to the available food in other parts of the plant subject to invasion by the fungus. Heald & Wilcox (2) grew *Diplodia zeae* on glucose agar, glycerine agar, and on sterile potatoes and carrots, but obtained no pyrenidial formation on these media. The fungus fruited on corn meal agar, sterile corn stalks glucose agar, and peptoneless agar acidified with citric acid. Corrosion of starch grains in the corn kernel is also

mentioned indicating the use of this substance by the fungus.

The writer has found *Diplodia zeae* to be an omnivorous feeder and growing well on a number of standard media. It also grows profusely and fruits on sterilized corn stalks, on cornmeal, oats, wheat, oatmeal and bean stems.

In order to obtain a better idea of what food constituents are preferred by the fungus, it was grown on synthetic media to which various carbohydrates and other substances were added. Using Pfeffer's solution as a base, M-10 solutions of the following sugars were made: sucrose, dextrose, levulose, maltose, and lactose.

Each of these solutions was put in seven flasks and sterilized and then planted with uniform small fragments of mycelium. The cultures were then held at room temperature for eight days, after which the fungus growths were dried to constant weight. The fungus grew well on these five sugars, making the least rapid growth on lactose.

In addition to growing the fungus on sugars, plantings were made on media as described by Crabill and Reed (5). On litmus cream agar a spreading growth and pink color reaction was obtained. Starch was not visibly digested by *Diplodia zeae*, but a good growth and profuse sporulation resulted. On peptone the growth was sparse and spreading with some sporulation. With asparagin and rosolic acid a slight reaction resulted. The dried powdered fungus gave a more pronounced color reaction, indicative of amidase activity. On amygdalin agar a very slight growth was obtained. Casein is digested by *Diplodia zeae*, the fungus growing and fruiting on the agar containing it. Egg albumin supports growth, but is not visibly digested by the fungus. Growth on inulin is very slight. On skimmed milk agar growth is profuse and the color change indicative of the action of erepsin is present. Pure cellulose agar induces profuse growth and sporulation.

Thus the ability of *Diplodia zeae* to use a wide range of food substance is apparent and indicates the production by the fungus of the enzymes lipase, erepsin, amidase, sucrase, maltase, invertase, and cytase and possibly trypsin and amylase.

The utilization of cellulose by the fungus is of particular interest, as the penetration and growth in the stalk and weakening of the nodes is thus more readily understood. The survival of the organism in old stubble and trash, even tho plowed under for several seasons, appears probable.

The destruction of cellulose by *Diplodia zeae* also has a bearing on methods of seed testing. In rag doll germinators

the fungus readily penetrates the cloth, attacking kernels of adjoining ears. The placing of paper next to the cloth, as in the Duddleson (6) "modified ragdoll," offers little more resistance to the fungus. *Diplodia zeae* readily penetrates the paper and will even grow on wet paper alone.

As there was some question whether the fungus could live and grow in the soil in a saprophytic manner, cultures were made on sterilized field soil, sterilized quartz sand and also on both of these to which sterilized organic matter had been added. The cultures were kept moist and held at room temperature. No growth was made on the sand which was free of organic matter. On both the sand and soil to which organic matter had been added, good growth resulted. The fungus invaded the sand and soil and was visible as white patches in the small cavities. On field soil an equally good growth occurred, the fungus apparently getting sufficient food material from the organic matter normally in the soil. This experiment suggests that the dry rot fungus may grow in a purely saprophytic manner and further emphasizes its ability to survive in the soil.

INFECTION STUDIES

In the past, studies of the infection of the corn plant by *Diplodia zeae* have been limited. Burrill & Barrett (1) were able to show the ability of the fungus to infect the ears, but were unable to successfully inoculate stalks or leaf sheaths except by stab inoculations. Heald and Wilcox (2), however, produced symptoms of the disease on stalk, husk and on the silks. They considered the latter manner of infection to be the typical means employed by the fungus in entering the ear. They also record that all ears are infected at the same time, which is a significant observation in the light of the method of infection and conditions governing it as given in the present discussion.

Smith (7) records brief experiments on soil infection and Van der Bijl (8) suggests the possibility of root infection by *Diplodia zeae*. However, infection of the corn plant in its various stages of development and the relation of environmental conditions to the invasion of the fungus has not received attention.

METHOD OF INFECTION

In an earlier paper (9 and 10) the writer called attention to the blotching of corn sheaths due to various organisms. Later observations have added to the list of agents that may cause a blotching of the corn sheath, one of the

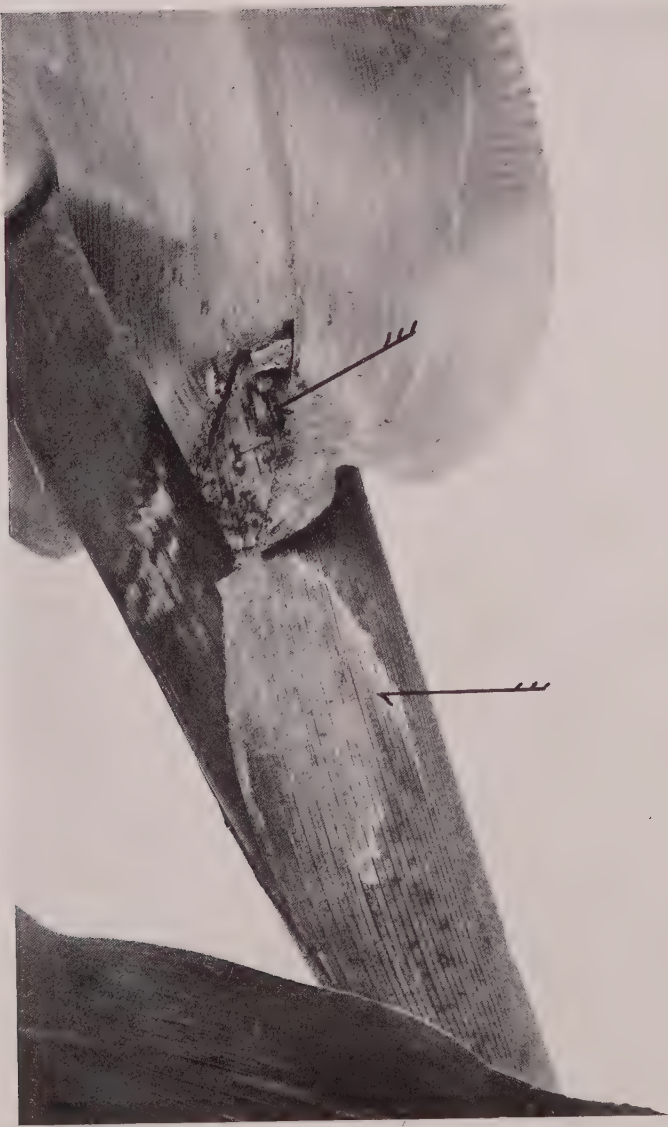


Fig. 9—The sheath and base of corn leaf showing the loosening from stalk. Note old pollen sacs inside sheath, also sugar deposited on outside of sheath.

most prevalent of which is *Diplodia zeae*. This is particularly significant in the present discussion as it explains the more prevalent method of infection of the corn plant by this fungus.

The *Diplodia zeae* may enter thru the silk, the chief method of infection may be described as follows: Prior to the production of flowers by the corn plant the ligules of the leaf sheath clasp the stalk very tightly, preventing anything from slipping down inside the sheath. If anything did drop down the rapid elongation of the stalks would carry it up again. After flowering, however, the stalk has ceased elongating and the action of the wind on the leaves has by this time loosened the ligule and exposed the cavity of the sheath (fig. 9). At the same time masses of pollen fall and roll down the leaf into the sheath, together with such spores as they may carry along or which are blown in, (fig. 10).

In addition to this combination of the spores and the stored food present in the pollen, a third factor of moisture enters. The sheaths of corn are frequently moist inside, even in dry weather holding condensed moisture in droplets (fig. 11), while in wet weather they stand full of water and often remain that way days after the rain. In 1922 there were periods when this condition existed to such an extent that the water became charged with sugar from the corn plant which was deposited on evaporation as a white crust at the edges of the sheath (fig. 9).

What better culture conditions for *Diplodia* spores could be described than the above combination of pollen, sugar, moisture? The fungus first feeds on the food material at hand. When this is exhausted it invades the corn plant (fig. 1, 15), causing spots and blotches on the upper part of the sheath, attacking the thin walled sheath cells rather than the thick walled cells of the stalk. At the base of the sheath the structure of the outer cells of the node (as shown in figs. 13 and 14) facilitates entry. There the fungus invades all tissues, confining its action, however, chiefly to those cells nearer the outer rind, discoloring the parts invaded, to a brownish, water soaked color. This discoloration is a good index of the presence of the fungus, as many isolations indicate. The fungus grows down from the node rather than up, tho traveling in both directions (fig. 12).

Infection of the ears occurs in the manner above described, infection entering at base of husk. However, a large percent of the infection on the ears is produced by the fungus working up the ear shanks from the diseased nodes.

The relation of sheath invasion is of much significance in *Diplodia* infection of corn, as shown by the following test. Each of five hills of corn was covered with a cheese cloth tent and the tassels removed from the stalks before they emerged. None of these plants showed any spotting of the sheaths until they were beginning to die and the tent had be-



Fig. 10—Mass of pollen sacs covered with *Diplodia zeae* lodged at leaf base. Midrib is killed in this way and fungus later works down or into node.



Fig. 11. Moisture held within leaf sheath. Picture taken the moment the sheath was cut away. Such moisture greatly adds in *Diplodia* infection.



Fig. 12. Section thru corn stalk showing darkened vascular bundles attacked by *Diplodia zeae* radiating from node.

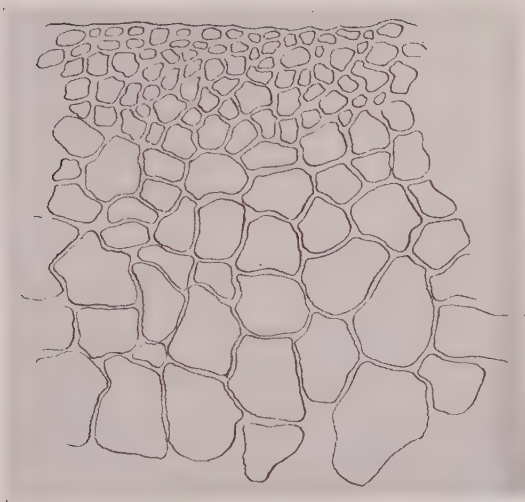


Fig. 13—Section of surface of corn stalk at node. The walls are thin compared to those at internode and more readily invaded by *Diplodia zeae*.

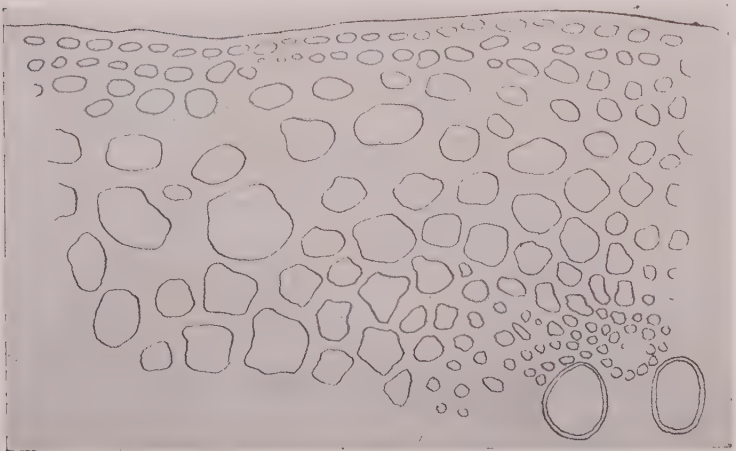


Fig. 14—Section of surface of stem at internode.

come weather beaten, while all surrounding corn was purple with blotches shortly after flowering. Plantings from these stalks, nodes and internodes, their entire length, showed no *Diplodia* or other infection, while surrounding uncovered plants were badly affected. The age of the plant does not necessarily influence infection, altho, as previously mentioned, strong, vigorously growing roots do not become infected and during rapid growth infection is excluded from the stem. However, in the latter case if the sheath is forcibly opened and *Diplodia zeae* inoculum introduced the plant will be attacked.

Partly because the lower leaf sheaths are older and are the first to open up and allow invasion and partly because of

the more constant moisture near the ground, *Diplodia zeae* is most profusely found on that part of the plant. This, perhaps, has suggested the idea that the fungus enters the crown and moves up the stalk, in some cases to the ear. Where these lower leaves are stripped off early in the season, very little *Diplodia* infection is found on the lower part of the stalk. In one locality in July, 40 plants were stripped of their three lower leaf sheaths. None of these stalks showed infection up to the fourth node, altho 11 out of an equal number of stalks, not stripped, were badly infected at the lower nodes as indicated by isolations from the inner tissues.



Fig. 15—*Diplodia zeae* invading corn stalk from growths on old pollen and pollen sacs.

LOCAL CHARACTER OF INFECTION

That *Diplodia zeae* is local and not systemic in its infection, gaining entrance at various nodes and on the ear rather than traveling up from the roots, is indicated by considerable evidence, as is shown in the following figures:

Using data from the described plots it is interesting to consider the relation of seed, seedlings and stalk infection to subsequent ear infection and also the distribution of moldy ears in the field.

The results are shown in the following table:

TABLE I—NUMBER OF DRY ROT EARS ON STALKS GROWN FROM DIPLODIA INFECTED AND CLEAN SEED

	Percent stand	Number of dry rot ears	Percent wt. dry rot ears
57 rows from clean seed	90	53	1.8
57 rows from <i>Diplodia</i> infected seed...	64	44	1.6

In table I. is given the percent stand resulting from planting seed attacked by *Diplodia zeae*. It will be seen that where ears attacked by this fungus are planted, a decrease of 26 percent of stand results. If *Diplodia zeae* followed this up by a systemic invasion of the remaining plots, a higher percent of dry rot ears would be expected in the diseased rows. This is not the case, however, for approximately the same number of moldy ears occur on all rows whether from diseased or clean seed. Infection on the ears appears to be generally distributed in the field as observations in other plots thruout the state also indicate. Further, the figures in table I. do not suggest that the less vigorous plants which result from diseased seed are any more subject to attack by *Diplodia zeae* than those from vigorous clean seed.

Supplementing the observations recorded in the above table numerous isolations were made from stalks attacked by *Diplodia zeae*. Large numbers of counts were made of diseased nodes on stalks in different fields. In the following tables this data is presented as additional evidence that infection by *Diplodia zeae* is local. Observations and counts on 984 stalks revealed the following:

4	percent of the stalks had	<i>Diplodia zeae</i> only at 1st node above ground.
8	percent of the stalks had	<i>Diplodia zeae</i> only at 1st and 2nd nodes.
22	percent of the stalks had	<i>Diplodia zeae</i> only from 1st to 3rd nodes.
16	percent of the stalks had	<i>Diplodia zeae</i> only at 2nd node — not above.
11	percent of the stalks had	<i>Diplodia zeae</i> only at 3rd node — not above.
8	percent of the stalks had	<i>Diplodia zeae</i> only at 4th node — not above.
1.7	percent of the stalks had	<i>Diplodia zeae</i> only at 5th node — not above.
.6	percent of the stalks had	<i>Diplodia zeae</i> only at 6th node — not above.

On 454 stalks in another locality the following infection was observed:

FREQUENCY OF DIPLODIA ZEAЕ AT DIFFERENT NODES

Nodes	1st Node	2nd Node	3rd Node	4th Node	5th Node	6th Node	7th Node	8th Node	9th Node
Percent of stalks attacked by <i>Diplodia zeaе</i>	58	66	63	47	29	14	5	1	.2

The inference to be drawn from the above is that *Diplodia zeaе* attacks chiefly the older lower part of the plant up to the first two or three nodes and rarely infects the younger parts above sixth or seventh node. On the same plants observed in the above data note was made of the relation of moldy and broken shanks to the attached ear, etc., with the resulting generalizations:

- Only 12 percent of broken shanks bore *Diplodia* infected ears.
- Only 12 percent of *Diplodia* ears were on *Diplodia* infected shanks.
- Only 19 percent of *Diplodia* rotted shanks were broken.
- Only 13 percent of *Diplodia* rotted shanks bore *Diplodia* infected ears.
- 48 percent of the *Diplodia* infected shanks were on clean stalks.
- 39 percent of the *Diplodia* infected ears were on clean stalks.
- 53 percent of the broken shanks were on *Diplodia* free stalks.

Further, the frequency of *Diplodia* rotted nodes below *Diplodia* rotted shanks was found to be as follows:

Node above ground.....	1	2	3	4	5	6	7	8
Percentage	22	26	19	24	17	13	8	2

It is evident from this summary of observations on nearly 1,500 plants that little relation exists between the *Diplodia* infection on stalk, shank and ear.

To more completely substantiate the above figures, plantings were made from numerous corn stalks, node by node, from the ground up. From one series of tests embodying 1,146 isolations, the following results were found:

- 8 percent of *Diplodia* infected nodes bore *Diplodia* free shanks.
- 5 percent of *Diplodia* free nodes bore *Diplodia* infected shanks.
- 31 percent of *Diplodia* internodes between infected nodes were free of *Diplodia*.
- 21 percent of internodes above infected nodes were free of *Diplodia*.
- 8 percent of all ear shanks were free of *Diplodia*.
- 50 percent of all cobs were free of *Diplodia*.
- 5 percent of nodes at shanks were free of *Diplodia*.
- 38 percent of internodes below *Diplodia* infected shanks were free of *Diplodia*.
- 10 percent of nodes below *Diplodia* infected shanks were free of *Diplodia*.

The frequency of occurrence of *Diplodia zeaе* at nodes and internodes in another set of observations is as follows:

TABLE II—PERCENT OF STALKS FREE OF DIPLODIA ZEAЕ AT NODES AND INTERNODES

	1st	1st	2nd	2nd	3rd	3rd	4th	4th	5th	5th	6th	6th	7th	7th
Node	7		0		11		15		18		12		0	
Internode		29		7		28		20		28		46		12

It may be noted in the above tabulations that a greater proportion of the infection by *Diplodia zeae* occurs at the nodes than at the internodes; also that the first node is not as frequently infected as the second and that the upper nodes and internodes are more free of *Diplodia zeae* than the lower ones. If the fungus travelled successively from node to internode up the stalk, plantings from node and internode would show the same degree of infection.

It also appears from these figures that clean shanks can be borne on infected nodes and infected shanks on clean nodes and that whereas only 8 percent of the shanks were free of *Diplodia* 50 percent of the cobs on these shanks were free. It might be mentioned that the isolations represented in these figures were from stalks that were mature and nearly ready to harvest at which time *Diplodia zeae* is most prevalent, rapidly invading tissue that has ceased to grow.

On greener stalks cut August 27, the results are somewhat different. From isolations from 50 stalks, made at every node and internode from ground up the following results were obtained:

TABLE III—INFECTION OF STALKS AT NODES AND INTERNODES

	Internode No. 1	Node No. 1	Internode No. 2	Node No. 2	Internode No. 3	Node No. 3	Internode No. 4
No. stalks showing infection at internodes	1		0		0		0
No. stalks showing infection at nodes....		1		3		2	

All sheaths in above stalks were covered with *Diplodia* sheath spot.

From another lot of somewhat more mature corn from another field the following data was gathered Sept. 1. This field it might be said, had borne corn the previous year and the soil was full of old *Diplodia* covered stubble. Tissue was plated from every node and internode from ground up to the fifth node using 28 representative stalks.

The outstanding points in these figures are that again the crown and first internode are less affected than those immediately above; also that in most of the stalks attacked gaps of clean tissue intervene between diseased areas. This point would have been evident in the data from the other tables had it been presented in the same way. Another noticeable

TABLE IV—DRY ROT INFECTION AT NODES AND INTERNODES

Stalk Number	Crown	Internode No. 1	Node No. 1	Internode No. 2	Node No. 2	Internode No. 3	Node No. 3	Internode No. 4	Node No. 4	Internode No. 5	Node No. 5	Condition of Stalk
1	0	*	*	*	*	0	0	0	0	*	*	dry
2	0	0	*	*	*	*	*	0	*	*	*	dry
3	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	0	0	0	0	0	0	0	0	
6	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	*	*	*	*	*	*	*	*	*	dry
10	0	0	0	0	0	0	0	0	0	0	0	
11	0	0	*	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	
13	*	*	0	0	*	*	*	*	*	*	*	dry
14	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	0	0	0	0	0	0	0	0	
16	0	0	0	0	0	0	*	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	
20	0	*	*	*	*	*	*	*	*	*	*	dry
21	0	0	0	0	0	0	0	0	0	0	0	
22	0	0	0	0	0	0	0	0	0	0	0	
23	*	*	*	*	*	*	*	*	*	*	*	dry
24	0	*	*	*	*	*	*	*	*	*	*	dry
25	0	0	0	0	0	0	0	0	0	0	0	dry
26	0	0	0	0	0	0	0	0	0	0	0	dry
27	0	0	0	0	0	0	0	0	0	0	0	
28	0	*	*	*	0	0	*	0	0	0	0	

* Diplodia infection.

thing in the above table is that certain stalks are very clean and others quite diseased. It is of particular interest in this connection to point out that the clean stalks were also green and the diseased stalks dry, that is those stalks still live and growing, are less subject to *Diplodia* attack than those that are on the decline.

Forty stalks, from those plants subjected to root inoculation on July 10 and Aug. 1, were also plated node by node Sept. 11 and 16. In these the same relation of green and dry stalks was manifest.

ON SEEDLINGS

Aside from the more obvious dry rot on the corn ears the infection of the seedlings is of particular importance. The questions arise, what is the relation of the diseased seed to seedling blight, can *Diplodia zeae* in the soil or on old stubble attack the seedling? With the first point under consideration, vigorous corn was taken from the germinator and transplanted before the plants were large enough to be injured. Fifty plants were planted in flats over corn stalks which were heavily covered with ripe pycnidia. The cultures were held at 28°C soil temperature for eight days, then dug

up. No effect was noticeable on the roots, tho these surrounded and lay next to the fruiting *Diplodia zeae* on the old stalks.

Later 46 clean plants were used, repeating the above experiment; of these five plants showed rotting of some of the very small roots directly adjoining the *Diplodia* covered stalks and 15 other plants showed a spot of decay here and there.

Further, 31 clean seedlings were planted in flats in which the soil was heavily seeded with *Diplodia* growing on sterilized oats and held at 30°C. Out of this lot 10 showed rotted roots and crowns. A similar lot of 30 seedlings was held for the same length of time at 15° to 20°C. In this case only five plants showed necrotic areas on the roots. The indications are, therefore, that *Diplodia zeae* can attack young corn roots. However, it must be considered that in the above experiments the amount of infecting material was very great and out of all proportion to what might occur in the field; also the soil temperature was higher than that occurring in the field, and more favorable.

Carrying the above experiment into the field, special infection plots were arranged in which corn showing 100 per cent germination was planted over *Diplodia* infected stalks, corn kernels, and masses of mycelium. The hills in these plots were planted one foot apart, three kernels to a hill, with the inoculum growing on oats placed below the seed corn. When plants were eight to ten inches high, every second and third hill was dug up and the seedling roots washed and examined. Three plantings were made, the first May 9, and later plantings May 16 and May 21. Of primary interest in these plantings was the stand; how many seedlings failed to emerge when given opportunity for infection.

The figures below indicate that in the field *Diplodia zeae* in proximity to germinating corn kernels may rot them or the young seedling so as to affect stand. In the case of *Diplodia* rotted seed, however, planted with healthy seed,

TABLE V—ACTION OF DIPLODIA ZEAЕ ON ROOTS OF CORN SEEDLINGS IN FIELD

Plot	No. of seed	Percent stand
Clean seed only.....	252	98
Clean seed over <i>Diplodia</i> infected kernels.....	252	98
Clean seed over mass of <i>Diplodia</i> inoculum.....	216	88
Clean seed over old <i>Diplodia</i> infected stalks.....	81	65

there is no indication of any appreciable spread and injury to the latter.

It must be remembered, however, that in the field, as in the greenhouse tests, the source of infection was in great excess of what would be found naturally and indicates the possibilities rather than what may ordinarily be found.

The main loss from seedling infection by *Diplodia zeae* arises from *Diplodia* infected seed. The kernels are attacked on the cob in varying degrees by the fungus resulting in dead or weak plants in the field. Field counts in different localities showed a loss in stand often as great as 25 percent. The results of such infection were most striking in test plots in which clean and diseased seed, as determined on the germinator, were planted side by side. Three plots were planted as follows: One plot near Story City on the farm of Clarence Johnson, consisting of 101, 25-hill rows, representing 101 ears of Johnson's corn. A second plot on the Agronomy Experiment farm at Ames, consisting of 201, 10-hill rows representing 201 ears of varying degrees of soundness from different parts of the state. A third plot located near the seed and consisted of plantings from 28 Iodent ears, 14 sound and 14 showing 1 to 4 *Diplodia* infected kernels. These ears were planted with 3 checks every seventh row, check No. 1 being seed from two sound ears shelled and mixed. Check No. 2 was a composite of all ears used in the plot and check No. 3 was a composite of all the diseased ears in the plot.

The whole of plot No. 3 was divided in 2 parts, planted at different dates. The first planting May 11 consisted of 43, 12-hill rows comprising plantings of the 28 ears and 5 sets of the checks as above described. The row numbers ran from east to west. To check on soil a duplicate of this planting was made the same date with rows numbering 1 to 43 west to east or just the reverse.

On May 22 the second part of plot number 3 was planted. It consisted of a replication of the planting on May 11th except there was one series of 24-hill rows numbering 1 to 43 east to west.

The following data gives the germination of the ears used in the plots above mentioned together with the resulting stand and seedling blight caused by *Diplodia zeae*:

In plot No. 3 from the 42 ears showing no *Diplodia* infection on the germinator a stand of 90 percent resulted in the field; while on the 42 rows showing 1 to 4 *Diplodia* infected kernels, only 62 percent stand was obtained. The 15 rows of checks from clean seed also gave 90 percent field stand; while the 15 rows of checks, representing a composite of all the *Diplodia* infected ears in the field, gave 66 percent.

It would appear from these figures that a germinator test may constitute an index of the *Diplodia* infection on the resulting seedlings. However, the question arises as to how many kernels from an ear must show *Diplodia* on the germinator in order to cause material loss of stand.

This relation of *Diplodia* infected kernels as found on the germinator and the resulting stand as determined with the 308 ears planted in plots 1 and 2 is as follows:

Percent of <i>Diplodia</i> infected kernels on germinator	Resulting stand
0	98%
12	86
25	80
37	76
50	45

These averages show a correlation between the number of *Diplodia* infected kernels on the germinator and the resulting field stand.

Individual exceptions to this are often noted however, as ears showing clean on the germinator may not give 100 percent stand in the field.

This is easily possible for dry rot often occurs on small groups of kernels on the ear not evident to the eye and in sampling such kernels may be missed.

PLANTS PAST SEEDLING STAGE

Second to the consideration of the effect on seedlings and stand is the action on plants after they have established themselves. In table VI. is given the percentage of plants having roots rotted in greater or less degree when grown over various types of inoculum.

The figures in table VI indicate that *Diplodia zeae* may rot the roots of young corn plants if supplied in sufficient quantities, however the experiment is hardly a criterion of what actually happens in the field for the inoculum was of great amount. The point of interest is not, however, how many plants had root lesions, but how many survived. Once the corn plant is independent of the food from the old kernel its rapidity of growth together with the unfavorable condi-

TABLE VI—ACTION OF D. ZEAЕ ON ROOTS OF MATURING PLANTS

Plot	No. Plants	No. plants showing rot on roots
Over <i>Diplodia</i> stubble	12	10
Over <i>Diplodia</i> culture	290	53
Over <i>Diplodia</i> kernels	60	22

tions for *Diplodia* development at that time, lessen the chances of rot infection. In the above experiment out of the hills planted one foot apart, every second and third was removed for observation, leaving a third of each lot, standing 3 feet apart. These plants grew the rest of the season in normal condition, many of them reaching over eight feet in height and producing good ears. If the roots sustained any injury from the inoculum it was not manifest. In fact, many plants that were dug later showed clean white roots growing thru old masses of dry rot inoculum without discoloration or injury. Isolations made from the crowns of 50 of these stalks, Aug. 27-31, showed *Diplodia zeae* in but one instance. The other 49 plants were clean and free of infection tho the seed had been planted in a mass of inoculum the size of a walnut. At time of digging some of this material still enveloped the crown of the plant.

In addition to the above observations on plants from seed planted in *Diplodia* inoculum, an experiment was made on healthy stalks as follows: On July 10 the soil was removed at one side of the base of 22 stalks, care being taken not to injure the roots. A mass of inoculum the size of an egg was tightly packed into the cavity between the roots below the crown of the plant and the soil was covered over it. On August 1, 18 more stalks were similarly treated. Table VII gives the results of these tests. Plants grown in a field containing old stubble covered with *Diplodia* were used as a check.

It must be said in regard to these figures that they represent isolations from the crown of the plant, which is relatively dead tissue when the plant is larger, and does not represent root infection. On none of the plants were root lesions found. These negative field trials suggest that root infection by *Diplodia zeae* was not significant under the conditions that prevailed in the field during 1922.

EFFECT OF *D. ZEA* ON MATURITY AND YIELD

In any field of corn toward the period of ripening some stalks will be found that mature sooner than others. Considering the prevalence of *Diplodia zeae* at this time on the low-

TABLE VII—RESULTS OF INOCULATING ROOTS AND CROWNS OF PLANTS WITH *DIPLODIA ZEA*

Date of inoculation	Date of isolation	Number of plants	Percent of <i>D. zeae</i> at crown
July 10	September 11	22	68
August 1	September 16	18	33
Natural soil infection	September 1	28	7



Fig. 16—Comparative height of corn from clean and *Diplodia* infected seed. Corn to left from clean seed, 4 feet high. Corn to right from *Diplodia* infected seed, a little over 3 feet high.

er nodes of the stalks the question arises does this infection play a part in the premature ripening. Counts, in one plot under observation showed that many early maturing plants had no *Diplodia zeae* at base, conversely many green growing stalks did show *Diplodia zeae* at the basal nodes.

Diplodia zeae, if it enters the plant, may no doubt hasten early ripening but it is doubtful if it is the cause. In table VIII are figures showing the relation of this maturity to *Diplodia* infection.

In the plots on which the above data were gathered rows 1, 11, 21, 31 and 41 were from the same seed and duplicated

TABLE VIII—RELATION OF MATURITY AND DIPLODIA INFECTION AT BASE OF STALK

Row No.	Plot No. 1		Plot No. 2		Average stalks per row	Average percent stand
	Planted	May 11	Planted	May 22		
	Number infected stalks	Number dying stalks Sept. 20th	Infected stalks	Dying stalks Sept. 20th		
1	19	9	7	1	69	96
11	25	6	3	0		
21	25	5	8	2		
31	13	3	7	3		
41	14	13	4	2		

on different dates 11 days apart. The degree of *Diplodia* infection recorded does not indicate a relation existing between the number of infected stalks and the number of dying stalks, moreover the dying stalks are not necessarily infected ones.

There is some evidence that infection by *Diplodia zeae* at the lower nodes of the stalk reduces yield. On 4080 plants observed those rows having an average of 13 stalks infected at base gave a yield of 22.6 lbs., while those rows having an average of 6 diseased stalks yielded 25.7 lbs. The stand in all rows was the same. While comparatively few plants are represented in these observations, the 2.6 lbs. greater yield on the less infected rows suggests that the invasion of *Diplodia zeae* into the lower nodes may be detrimental to the best functioning of the corn plant.

In addition to the early maturity and dying of individual stalks, the symptoms of barrenness and breaking of the stalk are of common occurrence in corn.

The prevalence of dry rot suggests that it may play a part in producing these symptoms. However, on the plants observed in one plot, 19 percent showed dry rot infection at the lower nodes, but only 3 percent of the plants were barren. It would seem that a greater amount of barrenness would occur if dry rot was an agent. Further no relation appeared to exist between disease seed and barrenness for the cleanest seed producing the most perfect stand of vigorous plants may produce more barren plants than badly diseased seed.

In the case of broken stalks a similar situation exists. Broken stalks are generally distributed over the field. The infection by *Diplodia* locally at the nodes is conducive to breaking. There is no relation between diseased seed and this breaking of the first few nodes above the ground as shown by records in over four thousand hills of corn.

In measuring the effect of dry rot on corn, yield must be the index of the injury.

In table IX are given the results from healthy and *Diplodia* infected kernels, expressed in yield. The figures represent data on 4,564 hills, planted three kernels to a hill.

TABLE IX—YIELD VERSUS GERMINATION AND STAND

	Percent germination	Total yield in pounds	Percent stand
57 rows of <i>Diplodia</i> free seed.....	100	1714	90
57 rows of <i>Diplodia</i> infected seed.....	75	1272	64

In six localities over the state similar plot comparisons show like results for *Diplodia* infected seed.

The bulk of this loss is no doubt due to lowered stand where infected seed is used, for it is quite obvious that a 64 percent stand would greatly decrease yield.

The effect of *Diplodia zeae* on seed and seedling while it constitutes the chief destruction of the fungus is not however, the only detrimental effect produced. The results of planting seed from dry rot infected ears indicate that the fungus not only kills a certain percent of the seed as evidenced by lower stand, but also the plants that do grow show weakening. In the following table is given comparative heights of plants from *Diplodia* infected and *Diplodia* free seed.

TABLE X—COMPARATIVE HEIGHT OF PLANTS FROM CLEAN AND INFECTED SEED

	Height in feet	Percent stand
45 rows <i>Diplodia</i> free seed.....	4.0	90
68 rows <i>Diplodia</i> infected seed.....	3.2	64

These figures indicate that the plants that do grow from diseased seed are in some way at a disadvantage and are stunted, differing in height by over one-half a foot from the plants from clean seed. Not only do the plants from seed from *Diplodia* infected ears show decreased height but they are more spindly and yellowish as well. In fig. 16 is shown the comparative height on July 11 of two of the rows included in the above table of measurements. The difference between the end and center of the pole held horizontally constitutes the difference in height between a row from healthy and infected seed. *Diplodia zeae* attacks the kernels of an ear in varying degrees, some are killed outright and microscopical examination shows such kernels to be completely overrun with the fungus mycelium. Other kernels are very slightly affected. This is the most probable explanation for the weak plants from dry rot infected ears. The badly infected kernels do not germinate, the slightly affected ones germinate but are weakened by the fungus.

CONTROL OF DRY ROT

The studies reported in this bulletin seem to warrant the following recommendations for the control of the dry rot disease; namely, a long rotation, early field seed selection and seed germination before planting in the spring. Since it has been found that the dry rot organism can live for at least three years on old corn stalks in the field, a four or five

year rotation is more desirable than a shorter one. Every effort should be made to hasten the decay of the stalks by thorough covering when the ground is plowed.

The seed corn should be selected as soon as the ears are well filled and dented without regard to the time of frost. Seed ears should only be selected from green standing stalks with sound shanks. After the seed has been gathered it should be cured in a dry, well ventilated place, not exposed to the weather.

Since it has been found that apparently sound ears may be slightly infected when gathered in the fall, and that the fungus may spread in the ear, while it is curing it is necessary to run germination tests in the spring before planting. The most practical methods of testing seed corn germination have already been described in Circular 78 of the Iowa Agricultural Experiment Station.

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